Annals of Gastroenterology (2014) **27,** 181-183

The microbiome in non-alcoholic fatty liver disease: associations and implications

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Title: Intestinal microbiota in patients with nonalcoholic fatty liver disease

Authors: Mouzaki M, Comelli EM, Arendt BM, Bonengel J, Fung SK, Fischer SE, McGilvray ID, Allard JP

Journal: *Hepatology* 2013;58:120-127

Title: Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease

Authors: Raman M, Ahmed I, Gillevet PM, Probert CS, Ratcliffe NM, Smith S, Greenwood R, Sikaroodi M, Lam V, Crotty P, Bailey J, Myers RP, Rioux KP

Journal: *Clin Gastroenterol Hepatol* 2013;11:868-875, e1-e3

Summary

Nonalcoholic fatty liver disease (NAFLD) is an increasingly significant public health problem as the risk factors for NAFLD such as obesity, insulin resistance and type II diabetes have also become more prevalent [1]. The composition of the intestinal microbiota (IM) has been previously implicated in the pathogenesis of obesity in clinical and animal studies, suggesting that obese humans exhibit a reduction in microbial diversity coupled with an increase in the ratios of *Firmicutes* to *Bacteroidetes* compared to lean humans [2,3]. Colonic bacteria may also have effects on the human host through intestinal absorption and delivery to the liver via the portal vein [4]. Therefore two recent papers examining the potential role of IM in NAFLD, by Mouzaki *et al* [5] and Raman *et al* [6] respectively, are both timely and relevant.

In the first of these, Mouzaki *et al* performed a prospective, cross-sectional study to determine whether differences in IM composition could contribute to the development of NAFLD [5]. Fifty total subjects constituted three groups of varying NAFLD disease progression: 11 simple steatosis (SS), 22 non-alcoholic steatoheaptitis (NASH), and 17 living liver donors as healthy controls (HC). Subjects in the SS group represent the mildest form of NAFLD, while the NASH group represents the most severe form and can lead to cirrhosis. Liver biopsy confirmed NASH, SS, or healthy liver in controls, and subjects in the NAFLD spectrum underwent extensive assessment to rule out other liver diseases. All subjects completed 7-day food and activity records, and provided stool samples for analysis. Quantitative real-time polymerase chain reaction measured microbial levels in stool samples from each subject. Levels of *Bacteroides/Precotella* (Bacteriodetes), *Clostridium leptum, C. coccoides,* bifidobacteria, *Escherichia coli*, total bacteria and Archaea were determined. Subjects in the NASH group had a lower ratio of Bacteriodetes to total bacterial counts in comparison to the SS or HC groups (P=0.006) and higher *C. coccoides* compared to the SS group (P=0.04). Interestingly, the authors were only able to detect Archaea in five HC, two SS and two NASH subjects, which limited the statistical power for any comparisons. After accounting for BMI and fat intake, no statistical difference remained in *C. coccoides* levels between groups, but there was still an independent significant association between subjects with NASH and a lower relative abundance of Bacteriodetes.

The authors concluded that these data provide evidence that the composition of the IM may play a role in the development of NAFLD. As microbes have established inflammatory roles, the authors also proposed that future studies may further elucidate the effects of the IM on the liver and the mechanisms underlying the development and progression of SS and NASH [5].

In the second study, Raman *et al* performed an observational case-control study that investigated the colonic microbiome and other volatile organic compounds (VOC) in

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Conflict of Interest: None

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Received 11 December 2013; accepted 12 December 2013

obese NAFLD patients and healthy controls [6]. Thirty obese NAFLD subjects and thirty healthy controls participated in the study. Neither group was subject to dietary restrictions, and the obese NAFLD participants had not undertaken any lifestyle modifications [6]. All provided stool samples for characterization of IM by multitag pyrosequencing and of VOC profiles by gas chromatography-mass spectrometry. In contrast to the results of Mouzaki *et al* [5], no discernable differences were found between the relative abundance of Bacteroidetes in obese NAFLD subjects vs. healthy controls. The researchers also did not find a correlation between BMI and the proportion of Firmicutes to Bacteroidetes as previously described by Ley *et al* [7], although the authors did note that the subjects in their study mainly had class I obesity, whereas the subjects in Ley *et al* had class III and class IV obesity. The authors did find small but significant differences between obese NAFLD patients and controls at the family and genus levels, including an over-representation of Lactobacillus and selected members of phylum Firmicutes in NAFLD subjects, while members of Ruminococcaceae (genus: *Oscillibacter*) were significantly under-represented. However, it is possible that these differences were due to obesity rather than NAFLD, as the controls were not obese. The authors concluded that there is a shift in the fecal microbiome and a significantly altered fecal VOC profile in obese NAFLD subjects, and propose that, as hepatotropic or hepatotoxic factors, these microbial VOC metabolites may be related to NAFLD [6].

Opinion

Taken together, the results of these two studies support that the gut microbiome is altered in subjects with NAFLD, although further studies such as microbiota transplantation experiments would be required to conclusively determine which of the identified differences are causal as opposed to resulting from the effects of the disease state on the IM. There are several important differences between the two studies. First, Raman *et al* performed multitag pyrosequencing to characterize the fecal microbiome (within the limits of classification achievable with currently available databases, as discussed by the authors) [6], whereas the study by Mouzaki *et al* employed Q-PCR to detect levels of specific microbes [5]. Although the mean BMI in the NASH [5] and NAFLD [6] groups in the two studies was similar, the range of BMIs in the NASH group studied by Mouzaki *et al* (24.2-49.5) was greater than that of the NAFLD group studied by Raman *et al* (29-35). Additionally, the study by Mouzaki *et al* was limited to Caucasians, and also compared dietary intake and physical activity levels between groups and correlated these with microbial levels, which was not undertaken by Raman *et al*, who also enrolled subjects of all races [6]. Lastly, the diagnosis of NAFLD was made based on laboratory measurements coupled with sonographic findings in the study by Raman *et al* [6], whereas Mouzaki *et al* used laboratory measurements and histologic analyses of liver biopsies, and further stratified subjects as having SS or NASH [5]. These differences in study design make it difficult to directly compare the results of the two studies. Nonetheless, there is evidence for a BMI-independent association between decreased Bacteriodetes and NASH [5], and some of the differences at the family and genus levels identified by Raman *et al* may also be associated with NAFLD. It is interesting that in the study by Mouzaki *et al*, the authors were unable to detect Archaea in the majority of study subjects [5]. In humans, the predominant Archaea are members of the order Methanobacteriales (methanogens), with *Methanobrevibacter smithii* being the most prevalent [8-10], and these can typically be detected in 70% of 'normal' human subjects [8]. That Archaea were not detected in more subjects by Mouzaki *et al* may result from differences between study populations, and/or may also be reflective of differences in the isolation techniques used. There are particular difficulties to working with *M. smithii* and other Archaea – as anerobes, stool analysis may be difficult if specific conditions are not met, and the primers used can also be critical. In addition, we note that both of these studies used stool samples for their analyses. Although this is typical of the majority of studies of the gut microbiota, the small intestine is a far more important site for digestion and absorption. Small intestinal microbes influence digestion and absorption directly, producing enzymes that assist the host in utilizing non-digestible carbohydrates and host-derived glycoconjugates; in deconjugating; dehydroxylating bile acids, and in cholesterol reduction and the biosynthesis of vitamins [11-15]. Our group has recently demonstrated that there are significant differences in the microbial populations in the small bowel and colon (personal communication with Dr Mark Pimentel), findings which are also supported by other studies [16]. These findings illustrate the importance of studying the microbiome in the small bowel directly (by obtaining duodenal aspirates) rather than relying on stool. Nonetheless, these pilot studies provide evidence that alterations in the microbiome may contribute to the development of NAFLD, and larger studies that include obese controls followed by animal studies are warranted to further elucidate the potential contributions of gut microbes and their metabolites to the pathogenesis of NAFLD. With recent advances in sequencing technologies, it should be possible to perform next-generation sequencing analyses of larger numbers of NAFLD subjects, including both obese and non-obese controls, in order to delineate the role of altered microbial populations in the development and progression of this disease.

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